

Human caspase-1 (SEQ ID NO:)
Human caspase-13^a (SEQ ID NO:)
Human caspase-4 (SEQ ID NO:)
Human caspase-5 (SEQ ID NO:)
Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
Mouse caspase-11 (SEQ ID NO:)
conserved amino acids^b

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conserved amino acids^b

[illegible]

SGSEGNVVKLCSLEEAQR1WKQKSAE1YPIMDKSSRTRLALI1CNEEFDS1PRRTGAEVDI
 -GSAATL1K1CPHEEF1KLCKERA1E1YPI1KERKDRTRLALI1CNTEFDHMP1PRNGAALDI
 -ESTDAL1K1CPHEEF1RLCKERA1E1YPI1KERNNRTRLALI1CNTEFDHL1PRNGADFDI
 -ESTN1L1K1CPREEF1RLCKNHDE1YPI1KKEDRRRLALI1CNTKFDHL1PARNGAHYDI
 -----Q1YPVMEKERRTCLAS1NRNKEFN1Y1LHNRNGSELDL
 SEVQDTL1K1CPRDQFCK1TERAKE1Y1PVMEKEGRTRLALI1CNKKFDY1LDFDRNADTDI
 --SLNTL1K1CSPPEF1RLCREKTQ1E1YPI1KEANGNRTKRALI1CNTEFKHLS1RYGAKFDI
 :***: * * * * *

1/2

FIGURE 2

	1				50
hCaspase-12	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Ap	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Bp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Cp	MADEKPSXGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Dp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Ep	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Fp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Gp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Hp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Ip	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Jp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Kp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
	51				100
hCaspase-12	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSSALLE	IQGAQPSGKL
KW-Ap	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Bp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Cp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Dp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Ep	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~PSGKL
KW-Fp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Gp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Hp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Ip	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Jp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Kp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSSALLE	IQGAQPSGKL
	101				150
hCaspase-12	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-ApIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-BpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-CpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-DpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Ep	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Fp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Gp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Hp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Ip	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-JpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Kp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
	151				200
hCaspase-12	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Ap	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Bp	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Cp	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Dp	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Ep	LDLLGGM*DLL	ENLGYSVVIK	ENLTA....
KW-Fp	LDLLGGM*DLL	ENLGYSVVIK	ESLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Gp	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Hp	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Ip	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLA
KW-Jp	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQEME.STFLV
KW-Kp	LDLLGGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV

L_a_aln

Human Caspase-12 compared to Mouse Caspase-12 with CARD domain, ICE-p20 domain, ICE-p10 domain and Active-site amino acids described.

hCaspase-12	<u>MADEKPSNGVLVHVMVKLLIKTFLDGI FDDLMENNVLNTDEIHLIGKCLKFVVSNAENLVD</u>	60
mCaspase-12	<u>MAARRTHERDPIYKIKGLAKDMLDGVFDDLVEKNVLNGDELLKIGESASFILNKAENLVE</u>	60
	** .:. : : : * * * :***:***:~*** **~ **~ .:~***:~	
hCaspase-12	<u>DITETAQIAGKIFREHLWNSKKQLS</u> -----	85
mCaspase-12	<u>NFLEKTD MAGKIFAGHIANSQEQLSLQFSNDEDDGPQKICTPSSPSES KRKVEDDEMEVN</u>	120
	:: *~***:~ *~***:~ ↑auto catalytic	
hCaspase-12	----- <u>SALLEIQGAQPSGKLKLC PHAHFHELKTKRADEIYPVMEKERRTCLALN</u>	134
mCaspase-12	<u>AGLAHESHLMLTAPHGLQSSEVDTLKLCPRDQFCIKTERAKEIYPVMEKEGTRLALI</u>	180
	: * :~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~	
	↑calpain ↑calpain	
hCaspase-12	<u>IRNKEFNYLHNRNGSEL DLLGMRDLENLGYSVVIKENLTAOEMETALROFAAHPEHOSS</u>	194
mCaspase-12	<u>ICNKKFDYLFDRDNADTDILNMOELLENLGYSVVLKENLTAOEMETELMQFAGRPEHOSS</u>	240
	* ~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~	
hCaspase-12	<u>DSTFLVFMSHGILNGICGTKHWDQEPDVLHDDTIFEI FNNRNCQSLKDKPKVIIMQACRG</u>	254
mCaspase-12	<u>DSTFLVFMSHGILEGICGVKHRNKKPDVLHDDTIFKIFNNSNCRSLRNKPKILIMQACRG</u>	300
	*****~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~	
hCaspase-12	<u>NGAGIVWFTTDSGKASADTHGRLLQGNICND AVTKAHVEKDFIAFKSSTPHNVSWRHETN</u>	314
mCaspase-12	<u>RYNGTIWVSTNKGIA TADTDEERVL SCKWNN SITKAHVETDFIAFKSSTPHNISWKVGKT</u>	360
	. * :~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~	
	↑auto catalytic	
hCaspase-12	<u>GSVFISQIIYYFREYSWSHLEE IFOKVOHSFETPNILTOLPTIERLSMTRYFYLFPGN</u>	373
mCaspase-12	<u>GSLFISKLIDCFKKYCWCYHLEE IFRKVOHSFEVPGELTOMPTIERVSMTRYFYLFPGN</u>	419
	~*:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~ *~***:~	

Legend for Domains as calculated by PFAM

CARD Domain xxxxxxx
ICE-p20 Domain yyyyyyy
ICE-p10 Domain zzzzzzz

Active-Site Residues: H...C

Calpain and Auto-catalytic cleavage sites determined for Mouse Caspase-12

FIGURE 3

H12_a_~1

CLUSTAL W (1.7) multiple sequence alignment

```

h_Caspase-3 -----
h_Caspase-7 -----
h_Caspase-12 -----MADE
m_Caspase-12 -----MAAR
h_Caspase-4 -----MAEG
h_Caspase-13 -----MAED
h_Caspase-5 -----MFKGILQSGLDNFVINHMLKNNVAGQTSIQTLVPNTDQKSTSVKKD
h_Caspase-1 -----MAD
h_Caspase-6 -----
h_Caspase-8 -----MDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPKIDALM
h_Caspase-10 MKSQGQHWYSSSDKNCKVSFREKLLIIDSNLGVQDVENLKFLCIGLVPNKKLEKSSSASD
h_Caspase-9 -----MDE
h_Caspase-2 -----MAADRGRIRILGVCGM
h_Caspase-14 -----

```

```

h_Caspase-3 -----
h_Caspase-7 -----
h_Caspase-12 KPSNGVLVHMVK---LLIKTFLDGI--FDDLMENNVNLNTDEIHLIGKCL-KFVVSNAEN
m_Caspase-12 RTHERDPIYKIK---GLAKDMLDGV--FDDLVEKNVLNGDELLKIGESA-SFILNKAEN
h_Caspase-4 N-HRKKPLKVLE---SLGKDFLTGV--LDNLVEQNVLNWKKEEEKKKYYD-AKTEDKVRV
h_Caspase-13 K-HNKNPLKMLE---SLGKELISGL--LDDFVEKNVLKLEEEEEKKKIYD-AKLQDKARV
h_Caspase-5 N-HKKKTVKMLE---YLGKDV LHGV--FNYLAKHDVLTLEKEEEKKKYYD-AKIEDKALI
h_Caspase-1 KVLKEKRKLFIR---SMGEGTINGL--LDELLQTRVLNKEEMEKVKREN-ATVMDKTRA
h_Caspase-6 -----
h_Caspase-8 LFQRLQEKRMLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRV
h_Caspase-10 VFEHLLAEDLLSEEDPFFLAELLYIIR-QKKLLQHLNCTKEEVERLLPTR--QRVSLFRN
h_Caspase-9 ADDRLLRRRCRLR-----LVEELQVDQLWDALLSSELFPHMIEDIQRAGSGSRDQARQ
h_Caspase-2 HPHHQETLKKNR---VVLAKQLLLSELLEHLLEKDIITLEMRELIQAKV--GSFSQNVE
h_Caspase-14 -----

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```

h_Caspase-3 -----MENTEN-----SVDSK-SIKNLEPKIIH-----GSE-----
h_Caspase-7 -----MADDQGCIEEQGVEDSANED-----SVDAPDRSSFVPSLFS-----KKKKN-----
h_Caspase-12 LVDDITETAQIAGKIFREHLWNS-----KKQLSSALL--EIQGAQ-----PSG-----K-----
m_Caspase-12 LVENFLEKTD MAGKIFAGHIANS-----QEQLSLQFSNDEDDGPQKICTPSSPSES KRKV
h_Caspase-4 MADSMQEQQRMAGQMLLQTFNID-----QISPNNKAHPNMEAGPP-----ESGES-----
h_Caspase-13 LVDSIRQKNQEAGQVFVQTFNLNID-----KNSTSIKAPETVAGPD-----ESVGS-----
h_Caspase-5 LVDSL R-KNRVAHQMFQTLLNMD-----QKITSVKPLLQIEAGPP-----ESAES-----
h_Caspase-1 LIDSVIPKGAQACQICITYICEEDS---YLAGTLGLSADQTS GNYLNMQDSQGVLS SFFPA
h_Caspase-6 -----MSSASGLRRGHPAGGE-----EN-----
h_Caspase-8 MLYQISEEVSRSSELRSFKLLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRV
h_Caspase-10 LLYELSEGIDSENKDMIFLLKDSLP-KTEMTSL SFLAFLEKQ GK---IDEDNLTCLEDL
h_Caspase-9 LIIDLETRGSQALPLFISCLEDTG---QDMLASFLRTNRQA AKLSKPTLENLT PVVLRP
h_Caspase-2 LLNLLPKRGPQAFDAFCEALRETKQGHLEDMLLTTLSGLQHVLPPLSCDYDLSLPFPVCE
h_Caspase-14 -----

```

FIGURE 4

h_Caspase-3	FESFSFDATFHAKKQIPCIVSMLTKE--LYFYH----
h_Caspase-7	FESQSDDPHFHEKKQIPCVVSMLTKE--LYFSQ----
h_Caspase-12	FET --- PNILTQLPTIERLSMTRYF -- YLFPGN ---
m_Caspase-12	FEV----PGELTQMPTIERVSMTRYF--YLFPGN---
h_Caspase-4	FET----PRAKAQMPTIERLSMTRYF--YLFPGN---
h_Caspase-13	FEK----PNVKAQMPTVERLSMTRYF--YLFPGN---
h_Caspase-5	FEV----PQAKAQMPTIERATLTRDF--YLFPGN---
h_Caspase-1	FEQ----PDGRAQMPTTERTVTLTRCF--YLFPGH---
h_Caspase-6	RVDFCKDPSAIGKKQVPCFASMLTKK--LHFFPKSN-
h_Caspase-8	VSN--KDDKKNMGKQMPQPTFTLRKK--LVFPSD---
h_Caspase-10	KRTVWG-AKQISATSLPTAISAQTPRPPMRRWSSVS-
h_Caspase-9	VSV-----KGIYKQMPGCFNFLRKK--LFFKTS---
h_Caspase-2	REGYAPGTEFHRCKEMSEYCSLTCRH-LYLFPGHPPT
h_Caspase-14	MAEAELVQEGKARKTNPEIQSTLRKR--LYLQ-----

Legend:

- ↓ Active-site Residues
- * Identical Residues
- : Conservative Substitution
- . Allowable Substitution

[illegible]

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h_Caspase-4          -----MAEGN-HRKKPLKLVLESLL
h_Caspase-5          MFKGILQSGLDNFVINHMLKNNAVAGQTSIQTLVPNTDQKSTSVKKDN-HKKKTVMKMLEYL
h_Caspase-13         -----MAEDK-HNKNP LKMLESLL
h_Caspase-12         -----MADEKPNSNGVLVHMVKLLL
h_Caspase-1          -----MADKVLKEKRKLFI RSM
                        : . . . .

h_Caspase-4          GKDFLTGVLDNLVEQNVLNWKEEEKKKKYDAKTEDKVRVMADSMQEKGQMAGOMLLQTFF
h_Caspase-5          GKDVLHG VFNYLAKHDVLTLEEEKKKKYDAKIEDKALILVDSLRL-KNRVAHQMFQTQLL
h_Caspase-13         GKELISGLLDDFVEKNVLKLEEEKKKKIYDAKLQDKARVLVDSIRQKNQEQAGQVFVQTFL
h_Caspase-12         IKTFLDGI FDDLMENNVLNTDEIHLIGKCLKFVVSNAENLVDDITETAQIAGKIFREHLW
h_Caspase-1          GEGTINGLLDEL LQTRVLNKEEMEKVKRENATVMDKTRALIDSVIPKGAQCACQICITYIC
                        : : *::: : : **.* . . . : *: . * :: :

h_Caspase-4          N-----IDQISPNNKAHPNMEAG--PPESGESTDALKLCP
h_Caspase-5          N-----MDQKITSVKPLLQIEAG--PPESAESTNILKLC P
h_Caspase-13         N-----IDKNSTS IKAPEETVAG--PDES VGSAATLKLC P
h_Caspase-12         N-----SKKQLSS--ALLEIQGA--QP-SGK----LKLCP
h_Caspase-1          EEDSYLAGTLGLSADQTS GNYLNMQDSQGVLS SFPAPQAVQDNPA MPTSSGSEGNVKLCS
                        : . . . . * :***.

h_Caspase-4          HEEFLRLCKERAEEIYP I KERNNRTRLALI ICNTEFDHLP PRNGADFDITGMKELLEGLD
h_Caspase-5          REEFLRLCKKNHDEI YPIKKREDRRRLALI ICNTKF D HLP ARNGAHYDIVGMKRLLQGLG
h_Caspase-13         HEEFLKLCKERAGEI YPIKERKDRTRLALI ICNTEFDHMP PRNGAALDILGMKQLLEGLG
h_Caspase-12         HAHFH ELKTKRADEI YPVMEKER T CLALNIRNKEFN YLHN RN GSEL DLL GM RD LLEN LG
h_Caspase-1          LEEAQRIWKQS AEIYPIMDK SSRTRLALI ICNEEFDS IPRTGA EVDITGMTMLLQNLG
                        . : . . ****: . . * *** * * :*: : *.*: *: ** **:*.

h_Caspase-4          YSVDVEENLTARDMESALRAFATR PEHKSS DSTFLVLMSHGILEGICGT V HDEKKPDVLL
h_Caspase-5          YT VVEKNLTARDMESVLRAFAARPEHKSS DSTFLVLMSHGILEGICGTAHKKKKPDVLL
h_Caspase-13         YTVEVEKLTARDMESVLWKFAAREEHKSS DSTFLVFM SHGILDGICGTMHSEEPDVLP
h_Caspase-12         YSVVIKENLTAQOMETALRQFAAHEHQSS DSTFLVFM SHGILNGICG TKHWDEPDVLH
h_Caspase-1          YSVDVKKNLTASDMTTELEAF AHRPEHKTS DSTFLVFM SHGIREGICGKKHSEQVPDILQ
                        *: * :*:*** :* : * * * : *:*****:***** :****. * . : *: *

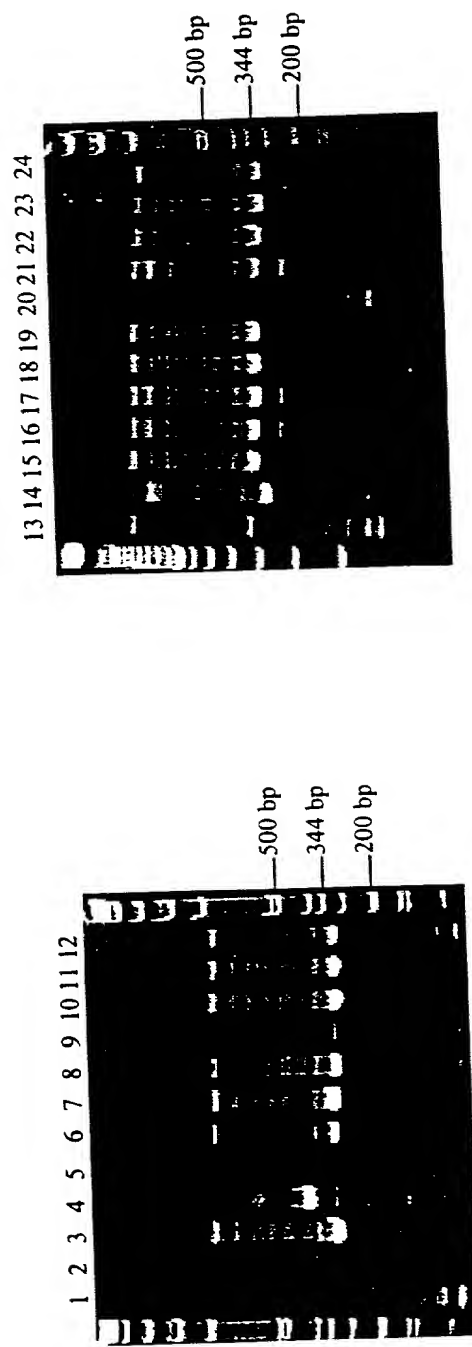
h_Caspase-4          YDTIFQIFNNRNCLSLKDKPKVII IQACRGANRGELWVR-DSPASLEVASSQSSE-NLEE
h_Caspase-5          YDTIFQIFNNRNCLSLKDKPKVII IQACRGEKHGELWVR-DSPASLAVISSQSSE-NLEA
h_Caspase-13         YDTIFRTFNNRNCLSLKDKPKVII IQACRGANRGELWVS-DSPPALADSFSQSSE-NLEE
h_Caspase-12         DDTIFEI FN NRNC QSLKDKPKVII IMQACRGNGAGIVWF TTDSGKASADTHGRLLQGNICN
h_Caspase-1          LNAIFNM LNTKNCP SLKDKPKVII IQACRGDSPGVVWFK-DSVGSGNLSLPTTE-EFED
                        ::* . :*.:** *****:***** * :*. ** : :

h_Caspase-4          DAVYKTHVEKDFIAFCSSTPHNVS WRDSTMGS IFITQLITCFQKYSWCCHLEEVFRKVQQ
h_Caspase-5          DSVCKIHEEKDFIAFCSSTPHNVS WRDRTRGS IFITELITCFQKYS CCHLM EIFRKVQK
h_Caspase-13         DAVYKTHVEKDFIAFCSSTPHNVS WRDIKKS LFITRLITCFQKYAWCCHLEEVFRKVQQ
h_Caspase-12         DAIVKAHVEKDFIAFKSSTPHNVSWRHETNGSVFI SQIIYYFREYSWSHHLEEIFOKVQH
h_Caspase-1          DAIKKAH IEKDFIAFCSSTPDNVSWRHPTMG SVF IGRLIEHMQEYACSCDVEEIFRKVRF
                        *: : * * ***** *****:***** . **:* :* :*: : . : *:**: *

h_Caspase-4          SFETPRAKAQMPTIERLSMTRYFYLFPGN
h_Caspase-5          SFEVPQA KAQMPTIERATLTRDFYLFPGN
h_Caspase-13         SFEKPNVKAQMPTVERLSMTRYFYLFPGN
h_Caspase-12         SFETPNILTOLPTIERLSMTRYFYLFPGN
h_Caspase-1          SFEQPDGRAQMPTTERVTLTRCFYLFPGH
                        *** * :*:*** * :*:*** *

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Tissue Profiling of hCaspase-12



1. Brain 2. Heart 3. Kidney 4. Spleen 5. Liver 6. Colon 7. Lung 8. Small Intestine
9. Muscle 10. Stomach 11. Testis 12. Placenta 13. Pituitary 14. Thyroid gland
15. Adrenal gland 16. Pancreas 17. Ovary 18. Uterus 19. Prostate 20. PBL 21. Fetal brain 22. Fetal liver 23. Fat 24. Mammary gland

FIGURE 6

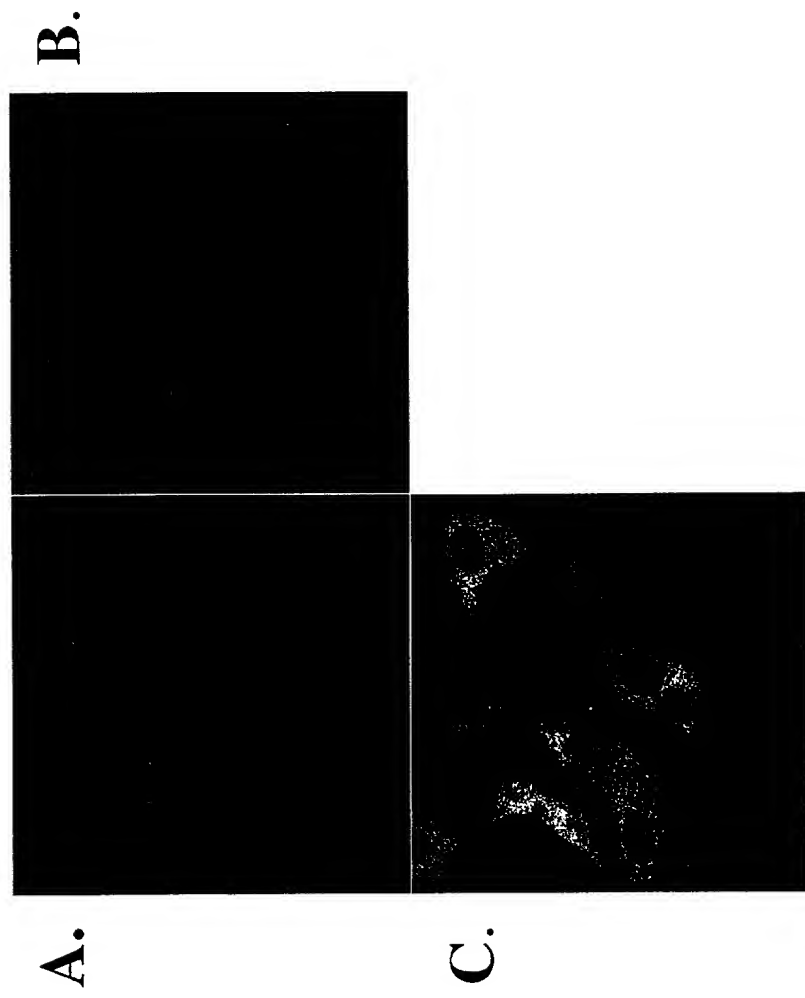


FIGURE 7

Cleavage of Procaspase-3 by hCaspase-12



FIGURE 8

SH-EP cell transfection +/- α -Fas

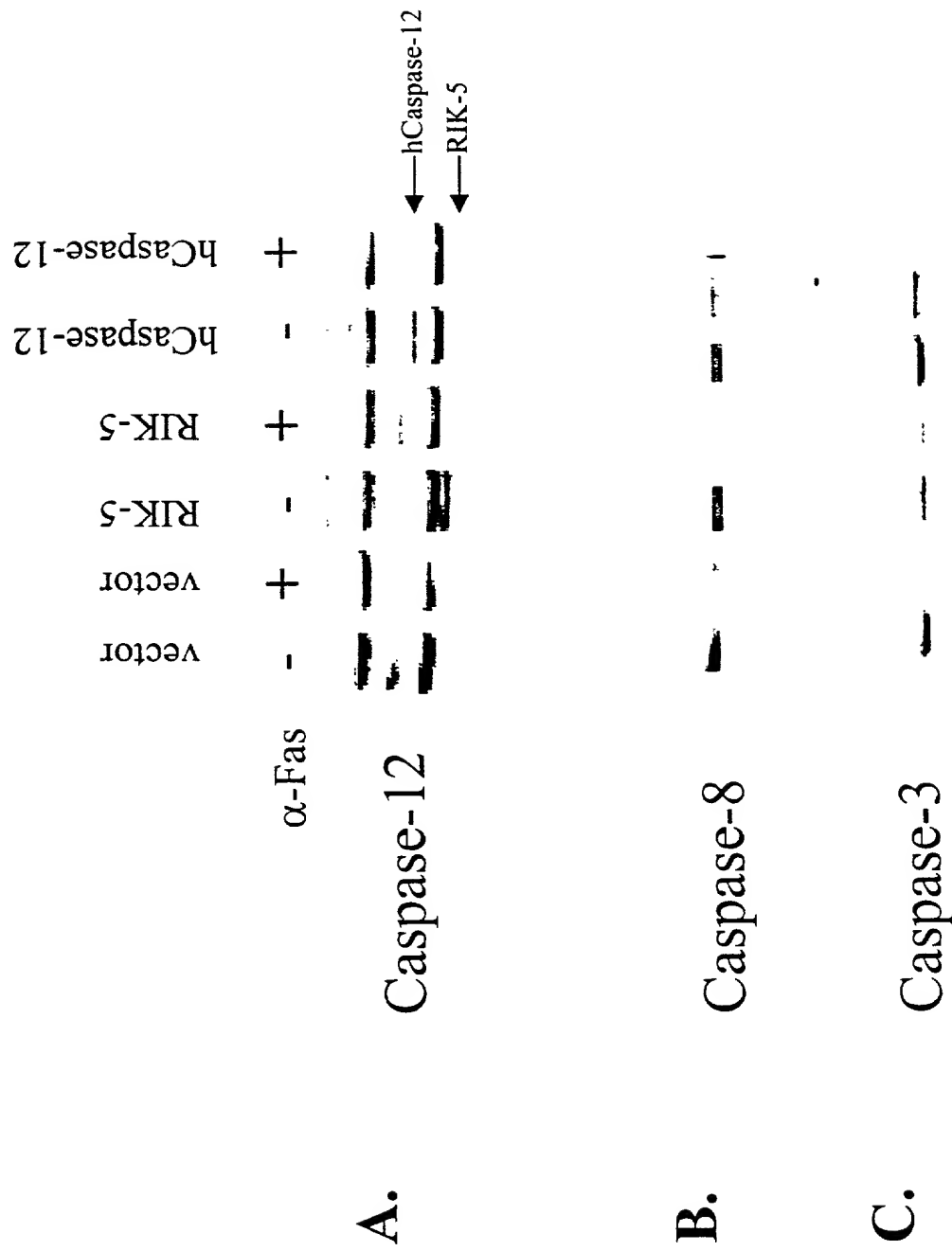


FIGURE 9

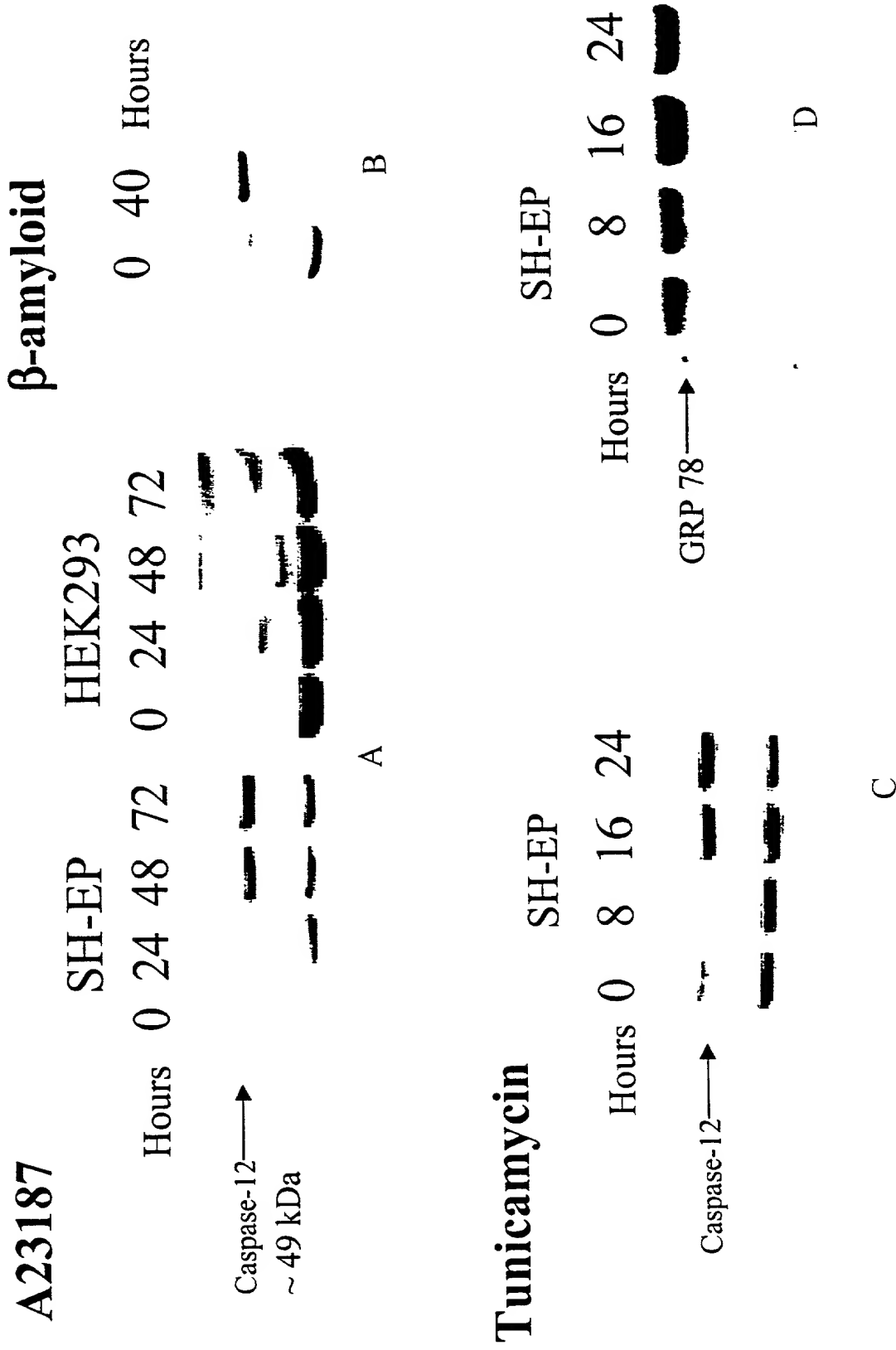


FIGURE 10

09561873 09561873

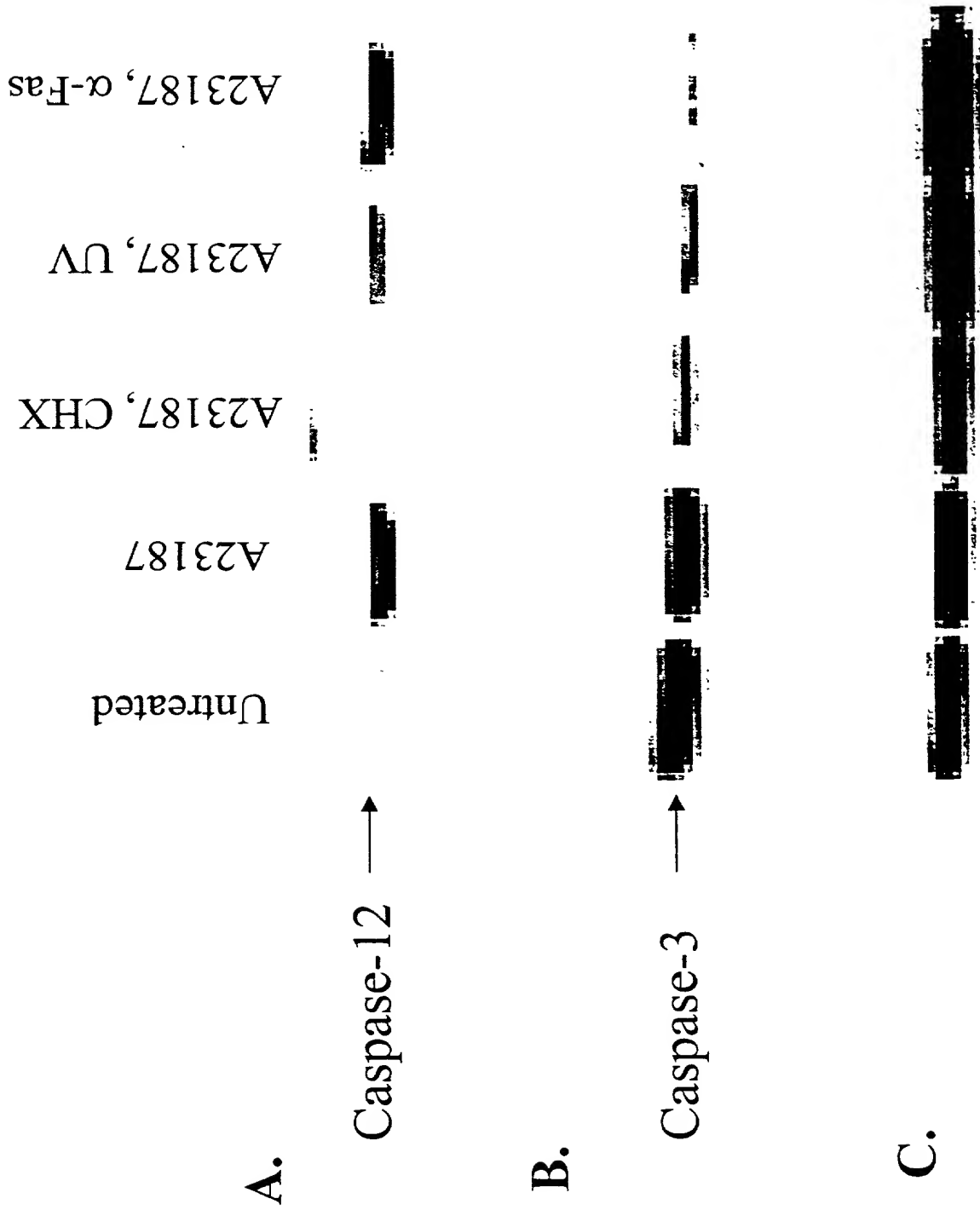


FIGURE 11

SH-EP cells treated with A23187, UV +/- inhibitors



FIGURE 12

Calpain cleavage of recombinant caspase-12

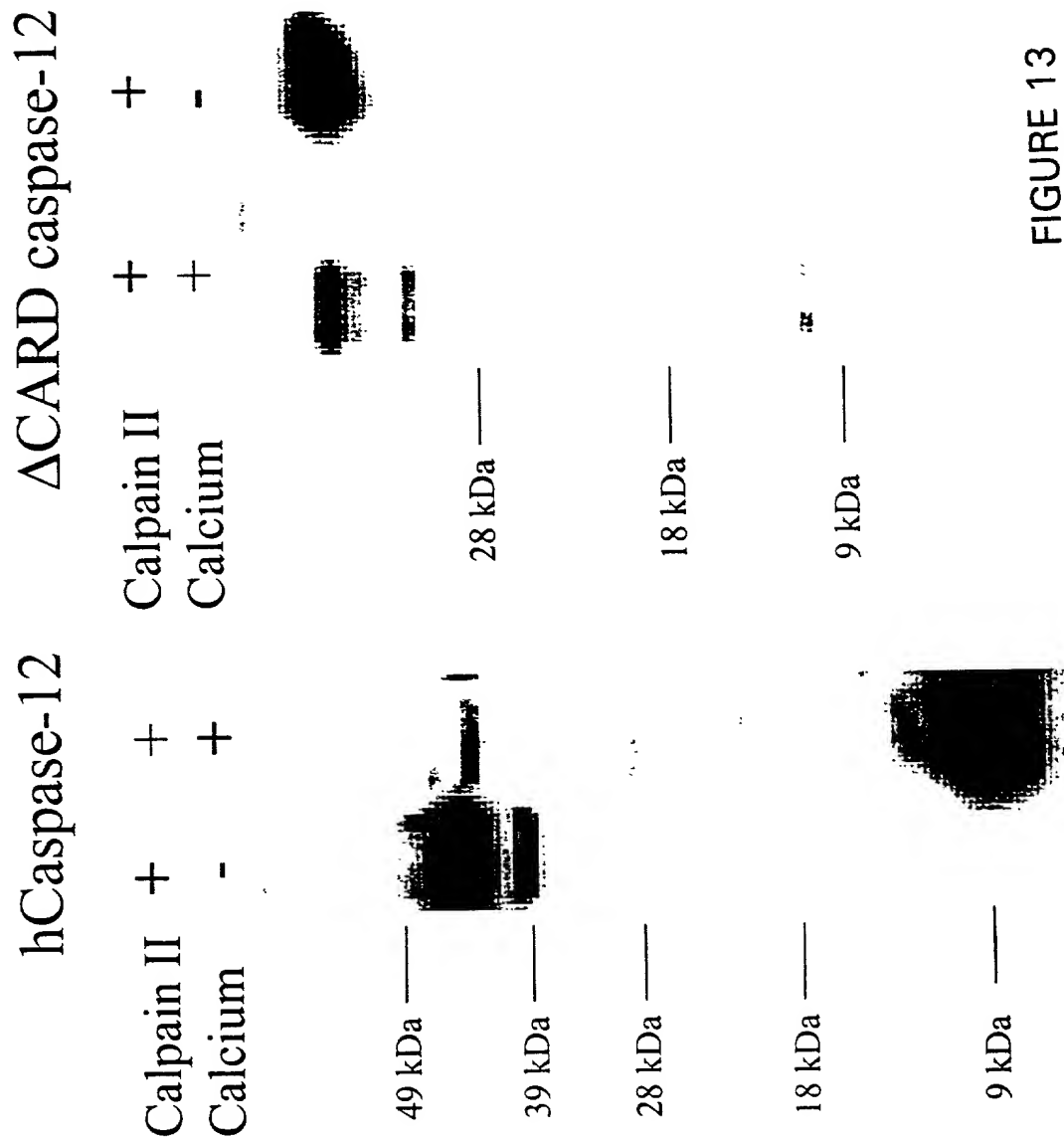


FIGURE 13